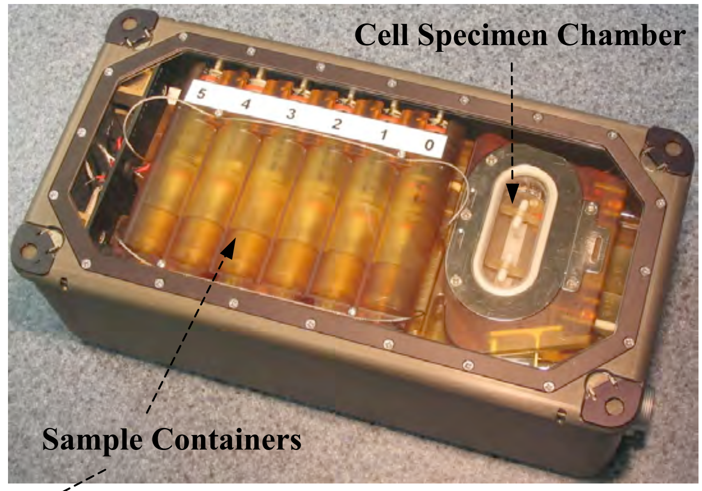
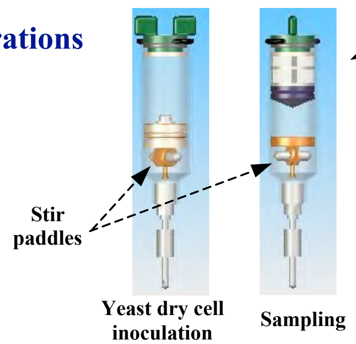
SINGLE LOOP for CELL CULTURE (SLCC)

Each SLCC provides:

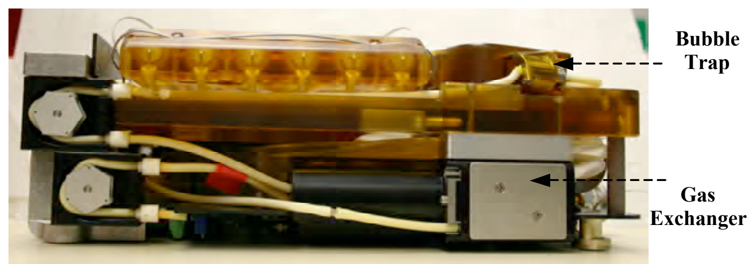
- 1 Cell Culture Perfusion Loop with a 10 mL Cell Specimen Chamber
- 6 Removable Sample/Inoculation Containers (provide containment of tox level 2 fixatives/additives)
- Fresh and Spent Media Bags
- CSC Stirring Capability
- Sample/Inoculation Container Mixing Capability
- Temperature and Humidity Data Recording
- In-line Bubble Trap
- External Viewing of CSC
- Autonomous Operations
- Gas Exchange
- Subculturing
- Crew Access



SLCC, Fully Assembled, top view



Sample Container Configurations



SLCC Fluid Loop without SLCC Box, side view

CGBA

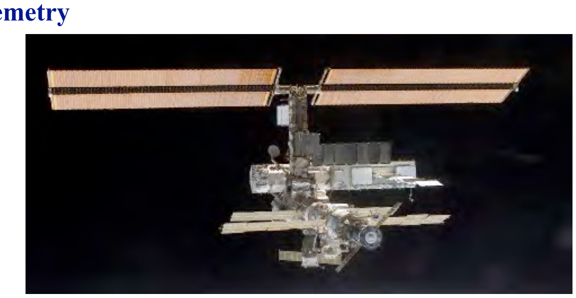
Thermal control provided by:

Commercial Generic Bioprocessing Apparatus (CGBA)

(Developed by BioServe Space Technologies)

CGBA capabilities include:

- 2 SLCC units supported at a time
- Temperature control range: -16^{o}C-37^{o}C
- Remote commanding
- Data telemetry



SLCC designed to support:

Science to answer bioastronautics roadmap questions

Ten SLCC flight units were delivered to NASA Ames Research Center in 2007 and are ready for flight.

Unique Capabilities of SLCC Hardware

A) SLCC is an uniquely designed perfusion-based system with independent control of:

- Mixing and cell suspension via stir paddles and fluid flow algorithms
- Gas exchange via the medium recirculation flow rate
- Nutrient/spent nutrient exchange via the nutrient re-supply rate

Independent control of SLCC operational parameters should enable experiments to separate:

The effect of gravity as a body force acting directly on cell structure

VS.

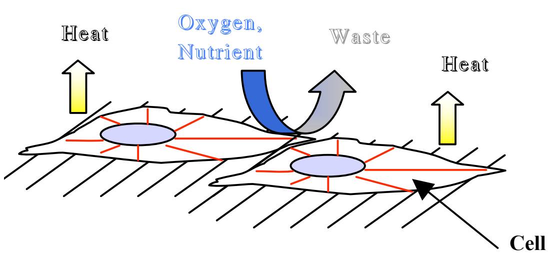
The effect of gravity acting to alter the mass transport environment around the cell

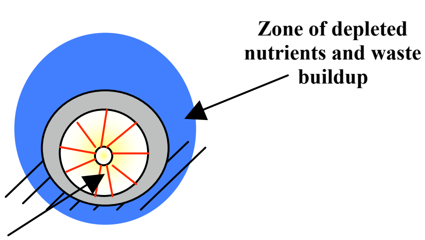
Normal Gravity

Convection drives exchange of nutrients and waste

Microgravity

No gravity= No convection Nutrient exchange is diffusion limited





B) SLCC automated functions:

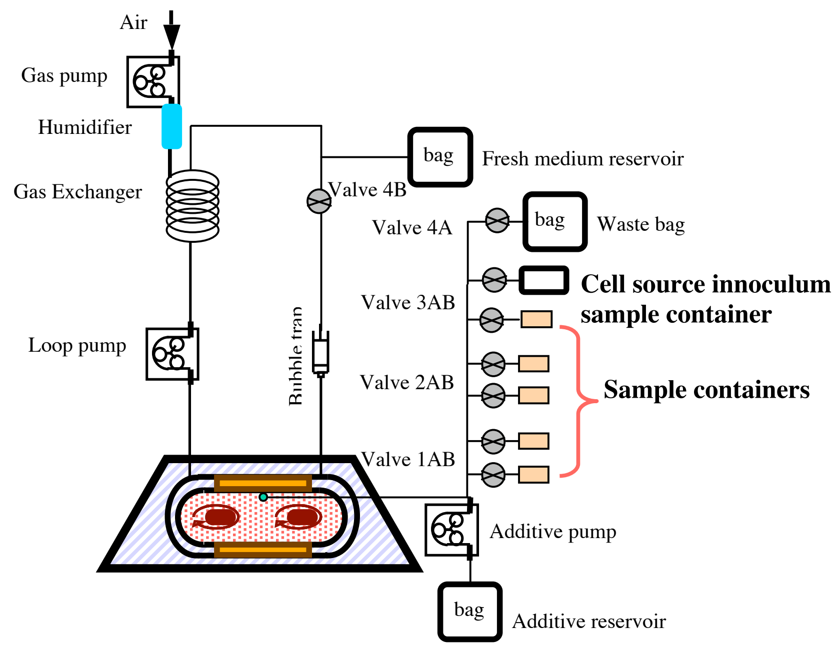
(Allow experiments to run without crew intervention)

- Cell sampling from the cell chamber
- Subculture
- Media and gas exchange
- Additive addition
- Mixing via stir paddles within the sample container
- Fixation of samples withdrawn from the cell chamber
- Initiation of suspension cultures on-orbit

C) Sample containers are replaceable on–orbit:

- Enables a high "n" and accommodates long duration experiments
- Enables crew to perform post experiment sample preservation procedures in a glovebox, e.g. cell drying

SLCC Fluid loop schematic, example



Cell Specimen Chamber Flow Characterization
(by Aurora, ARC, GRC)

NASA · Aurora FLIGHT SCIENCES · BioServe SPACE TECHNOLOGIES

Testing Goal:

- Evaluate the fluid flow environment inside the Single Loop for Cell Culture (SLCC) Cell Specimen Chamber (CSC)

Approach:

- Establish baseline data for environment in 200 mL Erlenmeyer flask on orbital shaker table - mixing, cell suspension, acceleration, shear.
- Evaluate CSC environment (with stir paddles rotating at 90 rpm) compared to shaker flask - mixing, cell suspension, acceleration, shear.

Methods used:

- ***Particle Imaging Velocimetry (PIV):*** Measures instantaneous 3D velocities of tracer particles in a plane. The tracer particles are illuminated in the specified plane by a pulsed laser sheet and their velocities are measured.

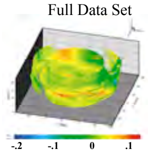
PIV Results: Testing of Shaker Flask and CSC

*Single plane of data shown (5.08 mm from bottom of 10 mL CSC)

PIV testing of Shaker flask

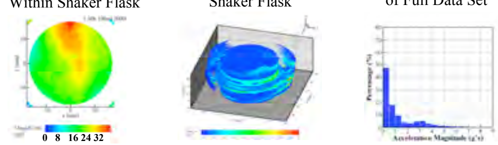
Velocity

Full Data Set



Acceleration

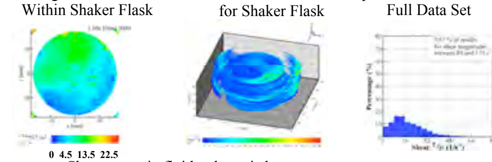
Single Data Plane Within Shaker Flask | Full Data Set for Shaker Flask | Population Distribution of Full Data Set



Most accelerations in fluid volume are fairly low.
Max acceleration measured ~70m/s^2 caused by free surface wave.

Shear

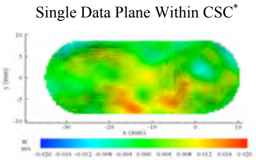
Single Data Plane Within Shaker Flask | Full Data Set for Shaker Flask | Population Distribution of Full Data Set



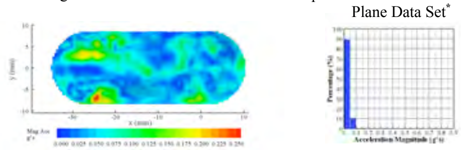
Shear stress in fluid volume is low.
Maximum effective shear stress measured was 3.74 x 10^{-4} N/m^2.

PIV testing of CSC

Single Data Plane Within CSC*

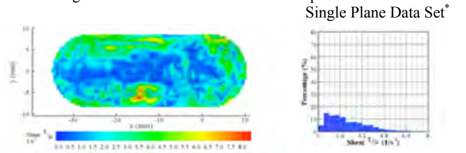


Single Data Plane Within CSC* | Population Distribution of Single Plane Data Set*



Acceleration magnitudes in the fluid volume for this data plane are low.
Peak accelerations measured in CSC are less than 1-g.

Single Data Plane Within CSC* | Population Distribution of Single Plane Data Set*



Shear stress in the CSC fluid volume for this data plane is low.
Peak shear values demonstrate very low maximum values.

Conclusions:

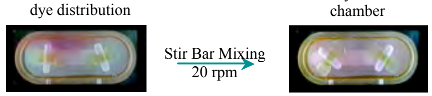
- Preliminary results from a single plane of data taken from within a CSC indicate a more benign flow field than the shaker flask flow field.
- Shear was not significant.
- Maximum accelerations in the shaker flask were higher than expected.

- ***Dye Front Flow Visualization:*** Assess flow distribution in the CSC by observing movement of dye introduced into a chamber and analyze dye residence time with a spectrophotometer.

Flow Visualization Results: Mixing, Suspension, and Flow Efficiency Characterization within the CSC

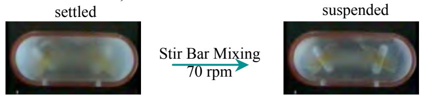
Uniformity of Nutrient/Oxygen Supply

Non-uniform dye distribution | Fully mixed chamber



Suspension Results with Cells

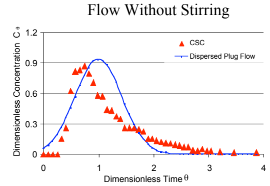
Tobacco Cells, settled | Tobacco Cells, suspended



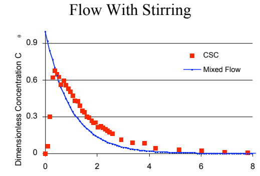
Flow Efficiency

Flushing performance of water into a dye filled Cell Specimen Chamber with and without stirring

Flow Without Stirring



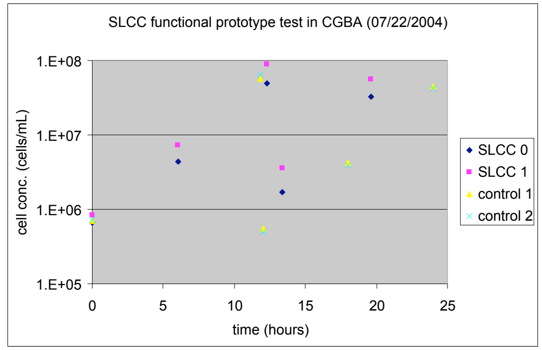
Flow With Stirring



Conclusions:

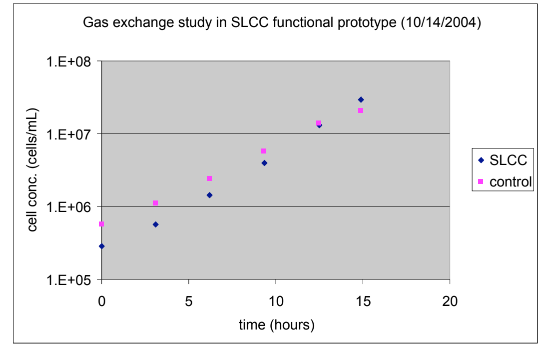
- Under conditions of flow and stirring, fluid flow in the chamber is dominated by stirring.
- Flow in the CSC without/with stirring corresponds to that of ideal plug/mixed flow.
- The need for efficient mixing and cell suspension in the CSC is met by the capability of stirring.

SLCC
Yeast Test Results



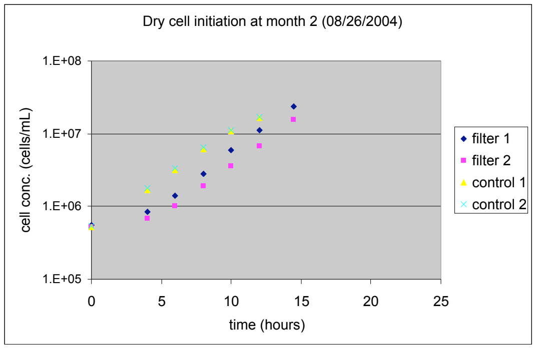
Cell Growth

Mission simulation test in SLCC functional prototype and CGBA



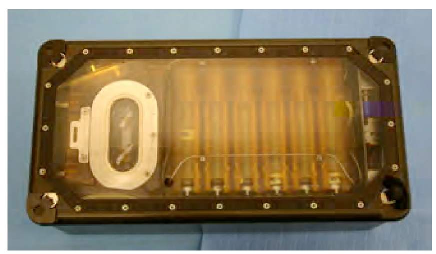
Gas Exchange

Experimental culture: 6 ml/min air circulation within SLCC*

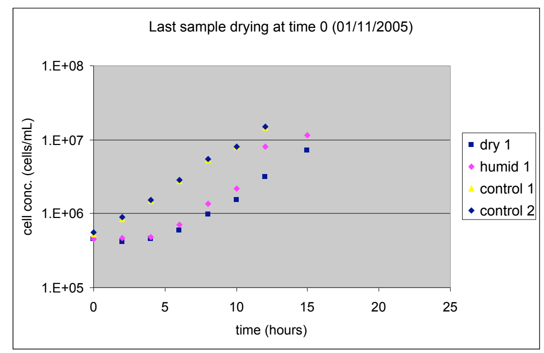


Dry Cell Initiation

Experimental culture initiated after cells were dried and stored on a filter for 2 months*

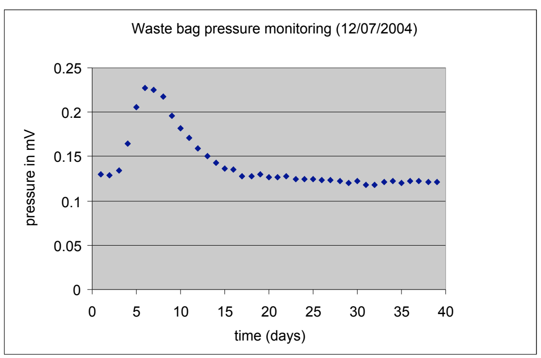


Cell Samples



Dried Sample Storage

Sample evaluation culture initiated after sample cells were dried *



Experiment Termination

Waste bag preloaded with 10mg sodium azide

All tests with:

***Saccharomyces Cerevisiae* Yeast Cells**

ATTC Wild Type (BY4743)

*All controls were grown in shaker flasks with fresh cells

Future Test

Samples stored in RNALaterII for DNA analysis

Next Generation SLCC-Mammalian Cells

Aurora has previously cultured a variety of mammalian cell cultures in hardware similar to SLCC.

Development Test Results with C2C12 Muscle Cells in CSCs

These results are from tests performed using single loop hardware with Cell Specimen Chambers (CSCs) at MIT/Aurora Flight Sciences.

TEST DESIGN

- Cells attach and grow in monolayer to confluence.
- Grown on tissue culture plastic (control) and Matrigel^{TM}-coated glass in CSC testing.
- Confluent cells differentiate to form myotubes.
- The myotubes are then stained with antibodies to tropomyosin to determine if differentiation occurred.

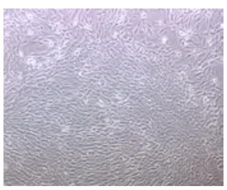
RESULTS

- Cells are viable, grow to confluency and differentiate.
- Myotube formation is slightly delayed in cells grown in CSCs as compared to controls.
- Tropomyosin expression is similar between CSC and control cultures.

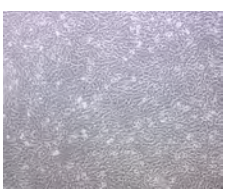
Methods

Media: Growth medium (GM): DMEM with phenol-red supplemented with 20% FBS and antibiotics. Fusion medium (FM) DMEM with phenol-red supplemented with 1% heat-inactivated HS and antibiotics.
Coating: Matrigel (MG) (dilution 1:1); thin gel method, 360 µl/CSC.
Seeding density: 2.0 x 10^5 cells/CSC (suspended in 2.2 mL GM, 22.7 cells/cm^2)
Feeding/Medium Exchange: GM for 48h, FM for the rest of the cultivation time. 100% medium exchange on days1(GM) and 2(FM)
Perfusion/Recirculation rate: Perfusion starts after 24h in GM. Periodic flow: 0.5 mL/min for 10 min every 1h.

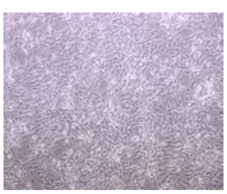
C2C12 Muscle Cells grown in CSCs and Controls have Comparable Cell Proliferation and Morphology at Day 2



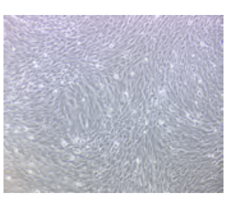
CSC 1



CSC 2



CSC 3

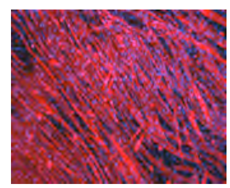


Static Well Plate

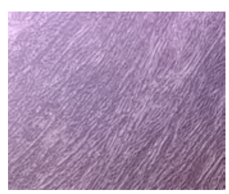
Differentiation at Day 10: CSC vs tissue culture plate control

Myotube Formation | **Tropomyosin Expression**

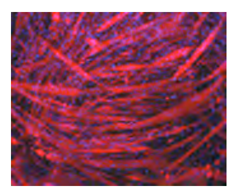
CSC 1



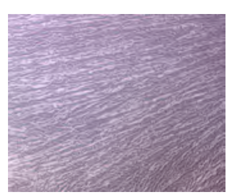
CSC 1



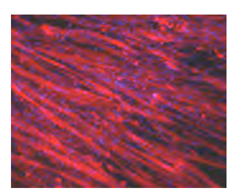
CSC 2



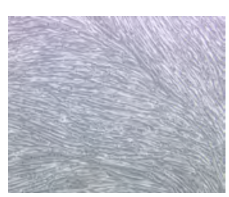
CSC 2



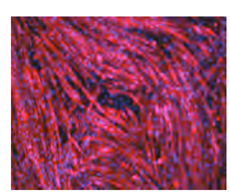
CSC 3



CSC 3



Static Well Plate



Static Well Plate